International Immunology, Vol. 15, No. 9, pp. 1073–1080
doi: 10.1093/intimm/dxg105

Human anti-idiotypic T cells induced by TCR peptides corresponding to a common CDR3 sequence motif in myelin basic protein-reactive T cells

Ying C. Q. Zang[1], Jian Hong[1,3], Victor M. Rivera[1], James Killian[1] and **Jingwu Z. Zhang[1–3]**

[1]Multiple Sclerosis Research Unit, Department of Neurology and Baylor-Methodist Multiple Sclerosis Center and [2]Department of Immunology, Baylor College of Medicine, Houston, TX 77030, USA
[3]Shanghai Institute of Immunology, Shanghai Second Medical University, Shanghai, China

Keywords: anti-idiotypic T cell, autoreactive T cell, multiple sclerosis, myelin basic protein, TCR

Abstract

T cells recognizing myelin basic protein (MBP) are potentially involved in the pathogenesis of multiple sclerosis (MS). *In vivo* clonal expansion of MBP-reactive T cells in MS may relate in part to dysfunction of peripheral regulatory mechanisms, including the anti-idiotypic immune network. In this study, we examined anti-idiotypic immune responses and the functional properties of anti-idiotypic T cells in patients with MS and healthy controls using TCR peptides corresponding to a CDR3 sequence motif preferentially expressed among T cells recognizing the 83–99 immunodominant peptide of MBP in some patients with MS. The study demonstrated that anti-idiotypic T cells could be induced *in vitro* by 8mer and 15mer peptides containing the CDR3 motif in MS patients and healthy controls respectively. The estimated precursor frequency of the anti-idiotypic T cells was slightly reduced in MS patients compared to control subjects. The obtained anti-idiotypic T cells recognizing the 15mer TCR peptide were found to express the CD4 phenotype, produce predominantly IL-10 and inhibit the proliferation of autologous T cells recognizing the immunodominant peptide of MBP. Anti-idiotypic T cells induced by the 8mer TCR peptide were predominantly $CD8^+$ cytotoxic T cells and exhibited cytotoxic activity against autologous MBP-specific T cells expressing the CDR3 sequence. When added in primary culture, both TCR peptides had a significant inhibitory effect on the T cell responses to the immunodominant peptide of MBP. The findings suggest that anti-idiotypic immune responses can be activated by selected TCR peptides and may play an important role in the *in vivo* regulation of MBP-reactive T cells.

Introduction

Autoimmune T cells are present in the normal T cell repertoire and are controlled by peripheral regulatory mechanisms (1–3). One of the important regulatory mechanisms involves an idiotype–anti-idiotypic network that interacts with idiotypic determinants of specific antibodies and T cells, contributing to the regulation of the immune responses (4,5). There is evidence both in experimental animals and in humans that anti-idiotypic T cells and antibodies regulate specific immune responses through the recognition of idiotypic determinants residing within the variable region or hypervariable region (CDR2 and CDR3) of TCR or antibodies (2,6,7). Anti-idiotypic T cells recognizing TCR framework regions were also reported (6). Although an idiotype–anti-idiotypic regulatory network has long been speculated to play an important role in the regulation of autoimmune responses, the functional and structural properties of anti-idiotypic T cells and antibodies remains unclear. The precursors of anti-idiotypic T cells and antibodies are found to pre-exist both in healthy and in some autoimmune conditions (8,9). However, the relationship of the precursors and their functional state with *in vivo* clonal

Correspondence to: J. Zhang, Department of Neurology, Baylor College of Medicine, 6501 Fannin Street, NB302, Houston, TX 77030, USA. E-mail: jzang@bcm.tmc.edu

Transmitting editor: L. Steinman

Received 27 August 2002, *accepted* 16 June 2003

expansion of autoimmune T cells seen in autoimmune conditions is unknown.

Multiple sclerosis (MS) is a demyelinating and inflammatory disease of the central nervous system with a presumed autoimmune pathology. There is increasing evidence that T cell responses to candidate myelin antigen(s), such as myelin basic protein (MBP), may play an important role in the pathogenesis of the disease (10–13). The pathogenic role of autoimmune T cells recognizing encephalitogenic epitopes of MBP is proven in EAE, an animal model for MS (14). There is evidence that T cells recognizing MBP undergo *in vivo* activation and clonal expansion in patients with MS as opposed to healthy or disease controls (12,15,16). It has been demonstrated that T cell responses to MBP are associated with preferential recognition of the 83–99 immunodominant region of MBP (MBP_{83-99}) (10–12). However, T cell recognition of these immunodominant regions of MBP was also detected in healthy individuals at relatively low frequency (10–12). In a recent study using CDR3 probe hybridization, T cells recognizing MBP_{83-99} were found to be as high as 1/300–1/1000 in peripheral blood mononuclear cells (PBMC) derived from MS patients (17). In addition, myelin-reactive T cells seem to occur at a significantly increased precursor frequency in the blood of MS patients at the time of clinical exacerbation as compared to that in remission (18). These autoreactive T cells may be activated in the periphery by viral or other microbial antigens through molecular mimicry (19). It is conceivable that persistent *in vivo* clonal expansion of myelin autoreactive T cells seen in MS may be associated with dysfunction of regulatory mechanisms, including an idiotype–anti-idiotypic network. However, the role of anti-idiotypic regulatory immune responses remains poorly understood.

This study was undertaken to investigate the functional properties and the precursor frequency of pre-existing anti-idiotypic T cells in relation to the T cell responses to MBP_{83-99} in MS patients and healthy individuals. Based on our observation in a recent T cell vaccination trial in which immunization with autologous MBP-reactive T cell clones was found to elicit anti-idiotypic T cell responses through interactions with CDR3 sequences of target TCR (8), we synthesized two TCR peptides corresponding to a previously identified common CDR3 sequence motif (LGRAGLTY) that is preferentially expressed among T cells recognizing MBP_{83-99} (20). The LGRAGLTY motif was detected in a significant proportion of MBP_{83-99}-reactive T cell lines derived from MS patients (20). In this study, two TCR peptides (8mer and 15mer) were used to activate pre-existing anti-idiotypic T cells, and analyze their precursor frequency in the blood of MS patients and healthy individuals using a cell culture-based frequency analysis to examine potential relationship between the precursor frequency of anti-idiotypic T cells and that of MBP-reactive T cells. The resulting anti-idiotypic T cell lines were further characterized for the phenotype, cytokine profile, reactivity pattern and cytotoxicity. Based on the identified regulatory properties of the anti-idiotypic T cells as described here, we further addressed whether the TCR peptides could be used to inhibit the induction of MBP_{83-99}-reactive T cells in primary PBMC culture. The findings described here are important for the understanding of the role of anti-idiotypic immune responses in the regulation of MBP-reactive T cells.

Methods

Reagents and peptides

Medium used for cell culture was Aim-V serum-free medium (Life Technologies, Grand Island, NY). Recombinant human IL-2 was purchased from Boehringer Mannheim (Indianapolis, IN). All peptides of human MBP and TCR were synthesized and purified by the Peptide Core Laboratory at the M. D. Anderson Cancer Center (Houston, TX). The purity of the peptides was >90%.

*Expression of DRB1*1501*

Total cellular RNA was extracted from PBMC specimens using the RNeasy Mini Kit (Qiagen, Santa Clarita, CA). RNA was reverse transcribed to first-strand cDNA using an Oligo-dT primer and the superscript pre-amplification system (Gibco, Gaithersburg, MD). cDNA was amplified by PCR using oligonucleotide primers specific for DRB1*1501 as described elsewhere (20). Briefly, 1 μl cDNA was added into the following amplification mixture: 5 μl of 10 × PCR buffer II (100 mM Tris–HCl, pH 8.3, 500 mM KCl), 3 μl of 25 mM magnesium chloride, 1 μl of 10 mM dNTP mix, 0.3 μl of Taq polymerase (5 U/μl) (AmpliTaq Gold, Perkin Elmer, Norwalk, CT), 10 pmol of a specific primer as the forward primer and 10 pmol of a specific primer as the reverse primer. The amplification profile used was 1 min at 95°C for denaturation, 20 s at 65°C for annealing and 40 s at 72°C for extension in a total of 30 cycles. The amplified PCR products were separated on a 1% agarose gel by electrophoresis and stained with ethidium bromide for visualization.

Detection of the LGRAGLTY sequence motif in MBP-reactive T cell lines

Briefly, cDNA products reverse transcribed from total RNA of each T cell line were analyzed by PCR using a corresponding 5′ Vβ forward primer and a 3′ Cβ reverse primer as previously described (20). The amplified PCR products were separated electrophoretically on a 1% agarose gel and transferred to a positively charged nylon membrane (Boehringer Mannheim, Indianapolis, IN) using vacuum blot (Bio-Rad, Hercules, CA) at 5 mHg for 90 min. DNA was fixed onto the membrane after exposure to UV cross-linking and prehybridized at 68°C for at least 1 h; 0.1 mg/ml of poly(A) was added to the prehybridization solution (5 × SSC, 1% blocking solution, 0.1% *N*-lauroylsarkosine and 0.02% SDS) to reduce non-specific binding of the probe to non-target DNA. Hybridization temperature and washing conditions were optimized to ensure stringent hybridization conditions. Hybridization was carried out in a buffer containing 5 × SSC, 1% blocking solution, 0.1% *N*-lauroylsarkosine, 0.02% SDS and a digoxigenin-labeled specific probe (0.3 pmol/ml) for 6 h. The detection of DNA hybrid products was performed using the Digoxigenin Luminescent Detection Kit according to the manufacturer's instructions (Boehringer Mannheim, Indianapolis, IN). The membrane was then exposed to X-ray film for 15–30 min at room temperature. The original T cell clone from which the LGRAGLTY sequence was derived served as a positive control and an unrelated CDR3 sequence was used as a negative control. Final CDR3 sequence assignment of the T

cell lines was confirmed by DNA cloning and sequencing as described previously (20).

Estimated precursor frequency of antigen-specific T cells in PBMC derived from MS patients and healthy individuals

To estimate the precursor frequency of T cells recognizing the TCR peptides or the MBP peptides (10,11,21), PBMC obtained from MS patients or healthy individuals were plated out at 200,000 cells/well in U-bottomed plates (Costar, Cambridge, MA) in the presence of selected peptides (40 wells for each peptide) at a concentration of 20 μg/ml. Seven days later, all cultures were re-stimulated with the corresponding peptide in the presence of irradiated autologous PBMC as a source of antigen-presenting cells (APC). After another week, each culture was examined for specific proliferation to the corresponding peptide in proliferation assays. Briefly, each well was split into four aliquots (~10^4 cells/aliquot) and cultured in duplicate in the presence of 10^5 APC pulsed with the corresponding peptide (a TCR or MBP peptide) or a control peptide. Cells were cultured for 72 h and pulsed with [^{3}H]thymidine (Amersham, Arlington Heights, IL) at 1 μCi/well during the last 16 h of the culture. Cells were then harvested and [^{3}H]thymidine incorporation was measured in a β-plate counter (Wallac, Turku, Finland). A T cell line was considered to be specific for the corresponding peptide when the c.p.m. was >1500 and exceeded the reference c.p.m. (in the presence of the control peptide) by at least 3 times. The precursor frequency of specific T cells was then estimated by dividing the number of positive wells by the total number of PBMC seeded in the initial culture. The same experimental conditions and the same calculation were used consistently throughout the study. Short-term T cell lines were analyzed for cytokine profile and selected T cell lines with high stimulation index were further characterized for the reactivity pattern and cytotoxicity.

Cytokine measurement

The cytokine profile of the resulting anti-idiotypic T cell lines was determined quantitatively using ELISA kits (PharMingen, San Diego, CA). Microtiter plates (96-wells; Nunc Maxisorp) were coated overnight at 4°C with 1 μg/well of a purified mouse capturing mAb to human cytokine [IL-4, IL-10, tumor necrosis factor (TNF)-α and IFN-γ] (PharMingen) in 100 μl of a carbonate buffer (100 mM, pH 9.5). Plates were washed 5 times with PBS (pH 7.0) containing 0.05% Tween 20 (PBS/T). Non-specific binding sites were saturated with 10% (w/v) FBS in PBS (FBS/PBS) for 1 h and washed subsequently with PBS/T. Supernatants and cytokine standards were diluted with PBS and added in duplicate wells. Plates were incubated at 4°C overnight and subsequently washed 5 times with PBS/T. Then 100 μl of the matched biotinylated detecting antibody (0.5 μg/ml for IL-4 and IL-10, and 1 μg/ml for IFN-γ and TNF-α) was added to each well and incubated at room temperature for 2 h. After washing, avidin-conjugated horseradish peroxidase (1:5000 dilution; Vector, Burlingame, CA) was added and plates were incubated for 1 h. Plates were then washed and 3,3′,5,5′-tetramethylbenzidine (Sigma, St Louis, MO) was used as a substrate for color development. The reaction was stopped by the addition of 1 N HCl. Optical density was measured at 450 nm by using an ELISA reader (Bio-Rad) and cytokine concentrations were analyzed quantitatively by microplate computer software (Bio-Rad) using a double eight-point standard curve. The detection limits for these cytokine measurements were said to range from 15 to 25 pg/ml.

Flow cytometry

To analyze the surface expression of CD4 and CD8 phenotypes, 10^5 cells of each T cell line were washed in PBS containing 1% FBS and 0.1% sodium azide (FBS/PBS) and resuspended in 100 μl FBS/PBS containing a 1:100 dilution of fluorochrome-labeled antibody [Simultest CD4–FITC/CD8–phycoerythrin, Leu-3a/2a; Becton Dickinson Immunocytometry Systems, San Jose, CA) or appropriate Ig isotype controls (γ2a–FITC/γ1–phycoerythrin; Becton Dickinson Immunocytometry Systems). After incubation for 30 min on ice, the cells were washed 3 times in FBS/PBS and fixed in 2% formaldehyde for flow cytometry assay. Cell preparations were analyzed using FACSCalibur (Becton Dickinson Biosciences, San Jose, CA).

Reactivity of anti-idiotypic T cell lines to TCR peptides

TCR-specific T cell lines (20,000 cells/well) were cultured with irradiated autologous PBMC (100,000 cells/well) as a source of APC in the presence or absence (medium alone) of corresponding TCR peptides (20 μg/ml). Cells were cultured for 72 h and pulsed with [^{3}H]thymidine (Amersham, Arlington Heights, IL) at 1 μCi/well during the last 16 h of the culture. Cells were then harvested using an automated cell harvester and [^{3}H]thymidine incorporation was measured in a β-plate counter (Wallac).

Inhibition assay

MBP_{83-99}-reactive T cell clones derived from MS-8, MS-9 and MS-10 or autologous T cells raised by phytohemagglutinin (PHA) activation were used as indicator cells and cultured at 20,000 cells/well in the presence of MBP_{83-99} peptide (20 μg/ml) or PHA (1 μg/ml), respectively, and irradiated autologous PBMC (100,000 cells/well) as a source of APC. Autologous CD4$^+$ anti-idiotypic T cells pre-activated and subsequently irradiated at 2000 rad were used as inhibitor cells and added at 20,000 cells/well to the wells containing autologous indicator cells. Cultures were kept for 72 h and pulsed with [^{3}H]thymidine (Amersham) at 1 μCi/well during the last 16 h of the culture. Cells were then harvested for the measurement of [^{3}H]thymidine incorporation as described above. Wells containing pre-irradiated inhibitor cells had a mean c.p.m. < 1200. The percent inhibition was calculated as 1 – (mean c.p.m. in the presence of inhibitor cells/mean c.p.m. in the absence of inhibitor cells) × 100%.

Cytotoxicity assay

Cytotoxic activity of selected CD8$^+$ anti-idiotypic T cell lines was measured in a lactate dehydrogenase-release assay according to the manufacturer's protocol (Promega, Madison, WI) (22,23). The E (anti-idiotypic T cells):T (MBP_{83-99}-reactive T cells or autologous T cells raised by PHA activation) ratio was 10. The experiments were performed in the presence and absence of two mAb to MHC class I (W6/32) and II (HB55) molecules at a concentration of 20 μg/ml. After incubation at

37°C for 4 h, supernatants were harvested and lactate dehydrogenase release was measured in an enzymatic assay. The maximum and spontaneous release was determined in wells containing detergent or medium alone. The percent specific cytolysis was calculated as (experimental release – spontaneous release/maximum release – spontaneous release) × 100%.

Inhibitory effect of the TCR peptides on the precursor frequency of MBP-reactive T cells

PBMC obtained from selected MS patients were plated out at 200,000 cells/well in U-bottomed plates (Costar) in the presence of selected peptides of MBP (40 wells for each peptide) at the concentration of 20 μg/ml. TCR_{15mer} or TCR_{8mer} peptide was added into the corresponding wells at a concentration of 20 μg/ml. Seven days later, all cultures were re-stimulated with the corresponding MBP peptides in the presence of irradiated autologous PBMC as a source of APC. After another week, each culture was examined for specific proliferation to the corresponding MBP peptide in proliferation assays as described elsewhere. The precursor frequency of specific T cells was then estimated by dividing the number of positive wells by the total number of PBMC seeded in the initial culture (10,11,21).

Results

Precursor frequency of T cells recognizing synthetic peptides containing a common TCR CDR3 sequence of MBP_{83-99}-specific T cells in patients with MS and healthy individuals

A group of 10 MS patients and a control group of 10 healthy individuals were included in the study. The clinical characteristics and the expression of DRB1*1501 are summarized in Table 1. Two peptides were synthesized, a short peptide of 8 amino acids in length (TCR_{8mer}) corresponding exactly to the LGRAGLTY sequence and a 15mer peptide (TCR_{15mer}) incorporating additional residues from the Jβ region (SSLGRAGLTYEQYFG). We first examined the precursor frequency of anti-idiotypic T cells recognizing the TCR peptides and its relationship with the precursor frequency of T cells recognizing the immunodominant region of MBP (MBP_{83-99}) and another MBP peptide ($MBP_{151-170}$) in patients with MS and healthy individuals. As shown in Fig. 1, T cells recognizing both TCR peptides could be detected in patients with MS as well as in healthy individuals. Anti-idiotypic T cells reactive to the TCR_{8mer} peptide occurred at relatively lower precursor frequency in MS patients than that in the control subjects (0.4×10^{-6} versus 0.6×10^{-6}). To a lesser extent, the precursor frequency of anti-idiotypic T cells recognizing TCR_{15mer} peptide was also decreased in patients with MS compared to that in control subjects (0.5×10^{-6} versus 0.7×10^{-6}). However, the differences in the T cell frequencies in this small sample size did not reach statistical significance. When plotted with the precursor frequency of T cells reactive to the MBP peptide, a trend for an inverse correlation between anti-idiotypic T cells recognizing the two TCR peptides and MBP_{83-99}-reactive T cells, but not $MBP_{151-170}$-reactive T cells, was noticed (Fig. 1). Interestingly, the decreased frequency of anti-idiotypic T cells seemed to correlate with an increased frequency of MBP_{83-99}-reactive T cells in MS patients examined (Fig. 1A and B).

Table 1. Clinical characteristics and the expression of DRB1*1501 in study subjects

Subject	Age	Sex	EDSS	Type of MS	DRB1*1501
MS-1	33	F	1.0	R/R	+
MS-2	55	F	6.5	R/R	+
MS-3	46	F	2.0	R/R	+
MS-4	60	M	7.0	S/P	+
MS-5	41	M	4.5	R/R	–
MS-6	48	F	8.0	S/P	–
MS-7	50	M	1.5	R/R	+
MS-8	53	F	1.0	R/R	+
MS-9	38	M	5.0	R/R	–
MS-10	46	F	1.5	R/R	–
NS-1	33	F	–	–	–
NS-2	34	F	–	–	+
NS-3	31	F	–	–	–
NS-4	32	F	–	–	–
NS-5	37	M	–	–	+
NS-6	44	F	–	–	–
NS-7	46	M	–	–	–
NS-8	63	F	–	–	–
NS-9	45	F	–	–	+
NS-10	40	F	–	–	–

The expression of DRB1*1501 was determined in PBMC specimens derived from MS patients and healthy individuals as described in Materials and Methods. EDSS, expanded disability score scale; R/R, relapsing-remitting MS; S/P, secondary progressive MS.

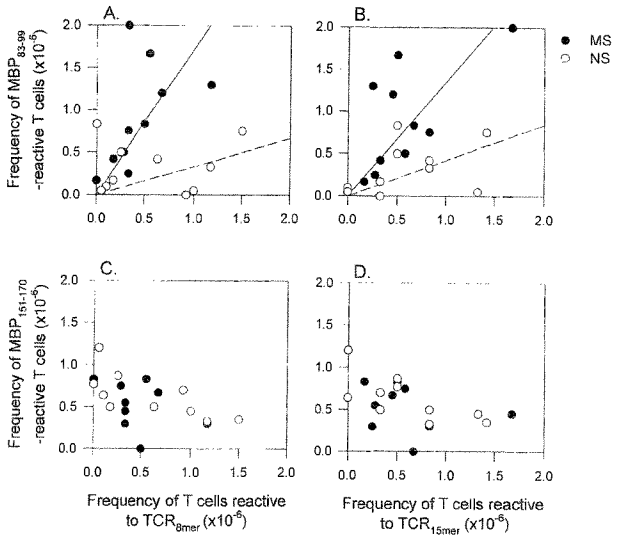

Fig. 1. The estimated precursor frequency of anti-idiotypic T cells recognizing the TCR peptides in relation to that of MBP_{83-99}-reactive T cells in patients with MS and healthy individuals. The precursor frequency of anti-idiotypic T cells recognizing the two TCR peptides (TCR_{15mer} and TCR_{8mer}) was estimated as described in Methods, and plotted with the precursor frequency of MBP_{83-99}-reactive T cells in the same MS patients (closed circles, n = 10) and healthy individuals (open circles, n = 10). The solid lines represent regression of the estimated T cell precursor frequencies in MS patients and the dashed lines represent those in healthy controls.

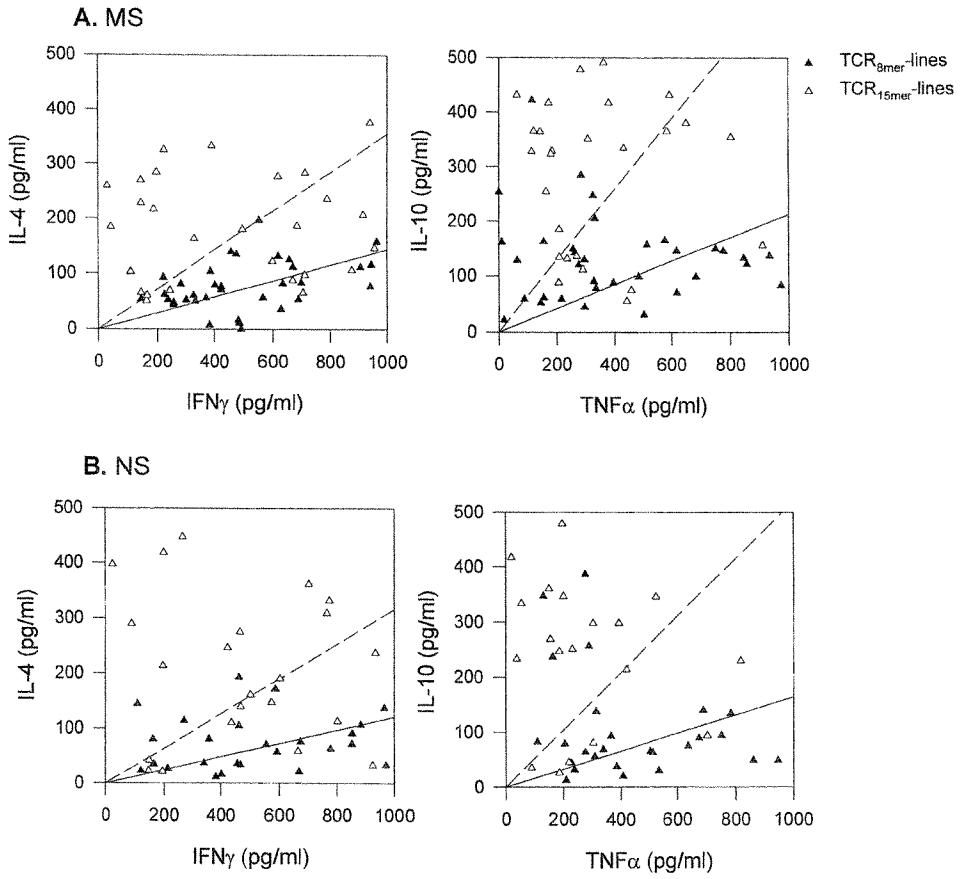

Fig. 2. The cytokine profile of anti-idiotypic T cell lines raised by the TCR peptides. A panel of 34 $CD8^+$ anti-idiotypic T cell lines recognizing TCR_{8mer} peptide (closed circles) and 29 $CD4^+$ T cell lines specific for TCR_{15mer} peptide (open circles) were analyzed for the production of the indicated T_h1 and T_h2 cytokines using ELISA kits. The obtained anti-idiotypic T cell lines were challenged with the corresponding TCR peptide, using medium alone as a control. Supernatants were collected at 48 h and assayed for the cytokines. The solid lines represent regression of the cytokine profile of $CD8^+$ anti-idiotypic T cell lines recognizing TCR_{8mer} peptide and the dashed lines represent that of $CD4^+$ anti-idiotypic T cell lines specific for TCR_{15mer} peptide. The differences in the regression of the cytokine profile between $CD4^+$ and $CD8^+$ anti-idiotypic T cell lines are statistically significant ($P < 0.01$).

Phenotypic and functional properties of anti-idiotypic T cell lines raised against the TCR peptides

The resulting anti-idiotypic T cell lines were then examined for phenotypic expression, cytokine profile and reactivity pattern. A panel of 34 T cell lines recognizing the TCR_{8mer} peptide and 29 T cell lines reactive to the TCR_{15mer} peptide were characterized first for the expression of CD4 and CD8. The majority of the T cell lines (29 of 34) recognizing the TCR_{8mer} peptide were of the CD8 phenotype as determined by >90% $CD8^+$ T cells, while all T cell lines recognizing the TCR_{15mer} peptide expressed the CD4 phenotype. The T cell lines were then analyzed for the production of the selected T_h1 cytokines (IFN-γ and TNF-α) and T_h2 cytokines (IL-4 and IL-10). As illustrated in Fig. 2, anti-idiotypic T cell lines recognizing the TCR_{8mer} peptide exhibited predominantly the T_h1 cytokine profile, producing mainly IFN-γ and TNF-α, but little IL-4 and IL-10, while T cell lines raised against the TCR_{15mer} peptide produced predominantly IL-10 and IL-4. There were no significant discrepancies in the cytokine production between the anti-idiotypic T cell lines derived from MS patients and healthy individuals.

A panel of representative anti-idiotypic T cell lines of both the CD4 ($n = 6$) and CD8 ($n = 6$) phenotypes was selected for further characterization, including the inhibitory (for $CD4^+$ T cell lines) and cytotoxic activity (for $CD8^+$ T cell lines). The selection of the T cell lines was based on high stimulation index (>10) in response to the corresponding peptides, but not the control peptide. Table 2 shows the reactivity of the selected anti-idiotypic T cell lines as well as three autologous MBP_{83-99}-reactive T cell clones (used as target/indicator cells) obtained from the same patients. The selected $CD4^+$ anti-idiotypic T cell lines were pre-activated with the TCR_{15mer} peptide and subsequently irradiated to prevent their interference in the proliferation assay. As shown in Fig. 3, $CD4^+$ anti-idiotypic T cell lines examined had an inhibitory effect on the proliferation of autologous MBP_{83-99}-reactive T cell clones and, to a lesser degree, the proliferation of the control T cells. In separate experiments, the $CD8^+$ anti-idiotypic T cell lines, with one exception, were found to exhibit significant cytotoxic

Table 2. The reactivity and phenotypic expression of MBP-reactive T cell clones and representative anti-idiotypic T cell lines used in the inhibition and cytotoxicity assays

T cell lines/clones	Phenotype	Reactivity (c.p.m. ± SEM)			LGRAGLTY motif
		Medium	MBP_{83-99} peptide	TCR peptide	
MBP-reactive					
MS8-G11	$CD4^+$	324 ± 12	4598 ± 212	–	Positive
MS9-C4	$CD4^+$	1277 ± 235	8723 ± 611	–	Positive
MS10-D8	$CD4^+$	1291 ± 89	7345 ± 182	–	Positive
Anti-idiotypic					–
MS8CD4-2	$CD4^+$	2840 ± 113	–	12277 ± 278	–
MS8CD4-5	$CD4^+$	1458 ± 86	–	6053 ± 102	–
MS9CD4-3	$CD4^+$	334 ± 12	–	3469 ± 87	–
MS9CD4-9	$CD4^+$	306 ± 23	–	7006 ± 154	–
MS10CD4-5	$CD4^+$	973 ± 16	–	3352 ± 99	–
MS10CD4-11	$CD4^+$	701 ± 20	–	2425 ± 110	–
MS8CD8-4	$CD8^+$	1598 ± 277	–	6458 ± 192	–
MS8CD8-12	$CD8^+$	1101 ± 47	–	7069 ± 612	–
MS9CD8-1	$CD8^+$	1907 ± 288	–	9113 ± 271	–
MS9CD8-6	$CD8^+$	743 ± 21	–	7665 ± 49	–
MS10CD8-1	$CD8^+$	1620 ± 109	–	7655 ± 290	–
MS10CD8-8	$CD8^+$	375 ± 17	–	5172 ± 131	–

The expression of the LGRAGLTY motif was analyzed using the method described in Methods. $CD4^+$ anti-idiotypic T cell lines were examined for the reactivity to the 15mer TCR peptide while $CD8^+$ anti-idiotypic T cell lines were tested under the same experimental conditions to the 8mer TCR peptide. The concentration of the peptides used in these experiments was 10 μg/ml.

activity towards autologous MBP_{83-99}-reactive target T cells, which could be blocked by the addition of a mAb to MHC class I molecules (W6/32) but not an antibody to MHC class II molecules (Fig. 3A).

Inhibitory effect of the TCR peptides on the induction of the T cell responses to MBP_{83-99} in PBMC obtained from MS patients

As the TCR peptides were demonstrated to activate pre-existing $CD4^+$ and $CD8^+$ anti-idiotypic T cells capable of regulating autologous MBP_{83-99}-reactive T cells, we further addressed whether the induction/activation of MBP_{83-99}-reactive T cells could be affected by the addition of the TCR peptides in primary PBMC culture. To this end, the TCR peptides were added into PBMC cultures that were stimulated either with the MBP_{83-99} peptide or with another MBP peptide (residues 151–170). PBMC specimens were obtained from three MS patients selected for positive detection of the TCR LGRAGLTY sequence motif. As illustrated in Fig. 4, the precursor frequency of T cells recognizing the MBP_{83-99} peptide, but not the $MBP_{151-170}$ peptide, was significantly decreased in cultures where the TCR_{8mer} peptide and the TCR_{15mer} peptide were added, while a control TCR peptide with an irrelevant TCR CDR3 sequence had no effect on the precursor frequency of MBP_{83-99}-reactive T cells. The TCR_{8mer} peptide seemed to have a greater inhibitory effect on the precursor frequency of MBP_{83-99}-reactive T cells.

Discussion

The study described here has yielded interesting findings that are important for the understanding of the role of anti-idiotypic immune regulation of MBP-reactive T cells in MS patients and healthy individuals, and has also raised some intriguing issues for future investigations. First, our T cell frequency analysis indicated that anti-idiotypic T cells recognizing a TCR CDR3 determinant(s) preferentially expressed by MBP_{83-99}-reactive T cells are pre-existing in both MS patients and healthy individuals, and can be induced by synthetic TCR peptides incorporating the LGRAGLTY sequence. There is a reverse correlation between the precursor frequency of anti-idiotypic T cells and that of T cells recognizing the immunodominant MBP_{83-99} peptide, suggesting the potential functional role of the anti-idiotypic T cells in the regulation of MBP_{83-99}-reactive T cells. The findings have raised further questions as to whether *in vivo* clonal expansion of MBP-reactive T cells often seen in MS patients (24,25) is associated, at least in part, with the frequency and the function of specific anti-idiotypic T cells. The issue should be addressed in great detail in future investigations. In addition to the idiotype–anti-idiotypic regulatory network, other peripheral immunoregulatory mechanisms are also found to play an important role in the regulation of MBP-reactive T cells in MS (3).

Second, TCR peptides of 8 and 15 amino acids seem to induce different T cell populations that can be differentiated by the phenotypic expression (CD4 versus CD8), cytokine profile (T_h1-like versus T_h2-like) and regulatory properties on MBP_{83-99}-reactive T cells (cytotoxicity versus inhibition). It is likely that $CD4^+$ anti-idiotypic T cells induced by the 15mer TCR peptide exert the inhibitory effect on MBP_{83-99}-reactive T cells by the production of IL-10, a known inhibitory cytokine. This possibility may explain the finding that when activated by the TCR peptide, the $CD4^+$ anti-idiotypic T cell lines examined also exhibited some inhibitory effect on the proliferation of control T cells. The functional properties of the $CD4^+$ anti-idiotypic T cells are consistent with those induced by immunization with different 15mer TCR peptides in patients with MS (26). Vandenbark *et al.* demonstrated in a clinical trial

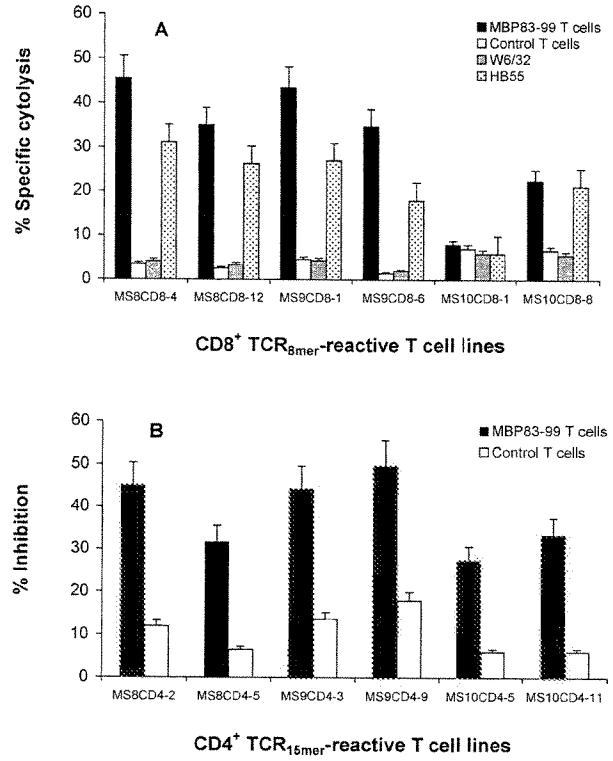

Fig. 3. The effects of the CD4+ and CD8+ anti-idiotypic T cell lines on autologous MBP_{83-99}-reactive T cell clones. (A) A panel of six representative CD8+ anti-idiotypic T cell lines recognizing TCR_{8mer} peptide was examined for cytotoxicity towards autologous MBP_{83-99}-reactive T cells; autologous T cells raised by PHA stimulation were labeled with ^{51}Cr as target cells. The E:T ratio was 10. The experiments were performed in the presence and absence of two mAb to MHC class I (W6/32) and II (HB55) molecules at the concentration of 20 μg/ml. Data are expressed as percent specific cytotoxicity. (B) In separate experiments, a panel of six representative CD4+ T cell lines specific for the TCR_{15mer} peptide were analyzed for an inhibitory effect on the proliferation of autologous MBP_{83-99}-reactive T cells and control T cells raised by PHA stimulation as described in Methods. The mean c.p.m. of the irradiated CD4+ anti-idiotypic T cell lines cultured alone was <1000.

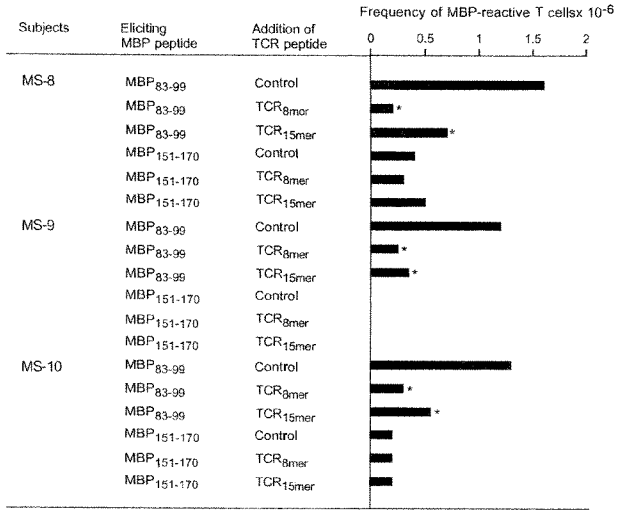

Fig. 4. The inhibitory effect of the TCR peptides on the precursor frequency of MBP_{83-99}-reactive T cells in selected patients with MS. The precursor frequency of T cells recognizing the 83–99 and 151–170 peptides of MBP was estimated in the presence and absence of the TCR peptides (20 μg/ml) respectively in PBMC that were obtained from three patients with MS (MS-8, MS-9 and MS-10) and expressed the LGRAGLTY motif. Asterisks indicate statistically significant differences ($P < 0.05$) between the precursor frequency of MBP-reactive T cells in the presence of the TCR_{8mer} and TCR_{15mer} peptides, and that in the presence of a mix of two control TCR peptides corresponding to the V–D–J junctional sequences (YSIRGQGN and ASSENRASYNEQFFG) of irrelevant T cell clones.

that synthetic peptides corresponding to CDR2 sequences expressed in some MS patients stimulated T cells that inhibited MBP-reactive T cells by producing IL-10 in culture (26). For CD8+ anti-idiotypic T cells, several existing models may explain how the idiotypic determinants of target TCR are presented to and recognized by anti-idiotypic T cells. There is experimental evidence indicating that peptides of cell-surface molecules are often presented by MHC class I molecules and that peptide-binding motifs for MHC class I molecules have been identified (27,28). Several recent studies have demonstrated that endogenous TCR peptides can be presented by self-MHC to anti-idiotypic T cells (29,30). It is also interesting to note that CD8+ cytotoxic anti-idiotypic T cells described here resemble those induced by immunization with irradiated autologous MBP-reactive T cells (T cell vaccination) in patients with MS (21,31). These CD8+ anti-idiotypic T cells induced by T cell vaccination preferentially recognize the CDR3 determinants of the immunizing MBP-reactive T cells and exhibit specific cytotoxic activity against the target T cells (8). The induction of the anti-idiotypic T cell responses in MS patients who received T cell vaccination coincided with the reduction and depletion of MBP-reactive T cells used for immunization (21,32).

In both studies discussed above, the induction of anti-idiotypic T cell responses by immunization, either with the TCR peptides or with irradiated autologous T cells, seemed to correlate with some clinical improvement in immunized MS patients (32,33). It is likely that anti-idiotypic T cells described here represent those that are induced by the different immunization approaches. Consistent with this possibility are the findings described here that the CDR3 peptides act as specific inhibitors for MBP-reactive T cells by inducing anti-idiotypic T cell responses. It remains to be tested in future investigations whether such TCR peptides may have therapeutic potential for a subset of MS patients in whom the common CDR3 sequence motif is expressed by MBP_{83-99}-reactive T cells that represent dominant MBP-reactive T cell populations.

Acknowledgements

The work was supported by the National Institutes of Health (NS41289 to J. Z.) and the Richardson Foundation.

Abbreviations

APC	antigen-presenting cell
MBP	myelin basic protein
MBP_{83-99}	immunodominant 83–99 peptide of MBP
MS	multiple sclerosis
PBMC	peripheral blood mononuclear cell
PHA	phytohemagglutinin
TNF	tumor necrosis factor

References

1 Cohen, I. R. 1992. The cognitive principle challenges clonal selection. *Immunol. Today* 13:441.
2 Kumar, V. and Sercarz, E. 2001. An integrative model of regulation centered on recognition of TCR/MHC complexes. *Immunol. Rev.* 182:113.
3 Jiang, H. and Chess, L. 2000. The specific regulation of immune responses by $CD8^+$ T cells restricted by the MHC class Ib molecule, Qa-1. *Annu. Rev. Immunol.* 18:185.
4 Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Inst. Pasteur)* 125C:373.
5 Lider, O., Reshef, T., Beraud, E., Ben-Nun, A. and Cohen, I. 1988. Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis. *Science* 239:181.
6 Zipp, F., Kerschensteiner, M., Dornmair, K., Malotka, J., Schmidt, S., Bender, A., Giegerich, G., de Waal Malefyt, R., Wekerle, H. and Hohlfeld, R. 1998. Diversity of the anti-T-cell receptor immune response and its implications for T-cell vaccination therapy of multiple sclerosis. *Brain* 121:1395.
7 Saruham-Direskeneli, G., Weber, F., Meinl, E., Pette, M., Giegerich, G., Hinkkanen, A., Epplen, J., Hohlfeld, R. and Wekerle, H. 1993. Human T cell autoimmunity against myelin basic protein: $CD4^+$ cells recognizing epitopes of the T cell receptor beta-chain from a myelin basic protein-specific T cell clone. *Eur. J. Immunol.* 23:530.
8 Zang, Y., Hong, J., Rivera, V., Killian, J. and Zhang, J. 2000. Preferential recognition of hypervariable region sequence by anti-idiotypic T cells induced by T cell vaccination in patients with multiple sclerosis. *J. Immunol.* 164:4011.
9 Hong, J., Zang, Y., Tejada-Simon, M., Li, S., Rivera, V., Killian, J. and Zhang, J. 2000. Reactivity and regulatory properties of anti-idiotypic antibodies induced by T cell vaccination in patients with multiple sclerosis. *J. Immunol.* 165:6858.
10 Ota, K., Matsui, M., Milford, E., Mackin, G., Weiner, H. and Hafler, D. 1990. T cell recognition of an immunodominant MBP epitope in multiple sclerosis. *Nature* 346:183.
11 Martin, R., Howell, M., Jaraquemada, D., Flerlage, M., Richert, J., Brostoff, S., Long, E., McFarlin, D. and McFarland, H. 1991. A MBP peptide is recognized by cytotoxic T cells in the context of four HLA-DR types associated with multiple sclerosis. *J. Exp. Med.* 173:19.
12 Zhang, J., Markovic, S., Lacet, B., Raus, J., Weiner, H. and Hafler, D. 1994. Increased frequency of interleukin 2-responsive T cells specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. *J. Exp. Med.* 179:973.
13 Steinman, L. 1996. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell* 85:299.
14 Zamvil, S. S., Nelson, P. Trotter, J., Mitchell, D., Knobler, R., Fritz, R. and Steinman, L. 1985. T-cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination. *Nature* 317:355.
15 Allegretta, M., Nicklas, J., Sriam, S. and Albertini, R. 1990. T cells responsive to myelin basic protein in patients with multiple sclerosis. *Science* 247:718.
16 Chou, Y. K., Bourdette, D., Offner, H., Whitham, R., Wang, R., Hashimm, G. and Vandenbark, A. 1992. Frequency of T cells specific for myelin basic protein and myelin proteolipid protein in blood and cerebrospinal fluid in multiple sclerosis. *J. Neuroimmunol.* 38:105.
17 Bieganowska, K. D., Ausubel, L., Modabber, Y., Slovik, E., Messersmith, M. and Hafler, D. 1997. Direct *ex vivo* analysis of activated, Fas-sensitive autoreactive T cells in human autoimmune disease. *J. Exp. Med.* 185:1585.
18 Tejada-Simon, M. V., Zang, Y., Yang, D., Hong, J., Li, S., Singh, R., Van den Berg-Loonen, E., Killian, J., Rivera, V. and Zhang J. 2000. Aberrant T cell responses to myelin antigens during clinical exacerbation in patients with multiple sclerosis. *Int. Immunol.* 12:641.
19 Hafler, D. 1999. The distinction blurs between an autoimmune versus microbial hypothesis in multiple sclerosis. *J. Clin. Invest.* 104:527.
20 Hong, J., Zang, Y., Tejada-Simon, M., Li, S., Singh, R., Yang, D., Rivera, V., Killian, J. and Zhang J. 1999. A common T cell receptor V–D–J sequence in Vβ13.1 T cells recognizing an immunodominant peptide of myelin basic protein in multiple sclerosis. *J. Immunol.* 163:3530.
21 Zhang, J., Medaer, R., Stinissen, P., Hafler, D. and Raus, J. 1993. MHC restricted depletion of human myelin basic protein reactive T cells by T cell vaccination. *Science* 261:1451.
22 Brander, C., Wyss-Coray, T., Mauri, D., Betten, F. and Pichler, W. 1993. Carrier-mediated uptake and presentation of a major histocompatibility complex class I-restricted peptide. *Eur. J. Immunol.* 23:3217.
23 Korzeniewski, C. and Callewaert, D. 1983. An enzyme-release assay for natural cytotoxicity. *J. Immunol. Methods* 64:313.
24 Wucherpfennig, K. W., Zhang, J., Witek, C., Matsui, M., Modabber, Y., Ota, K. and Hafler, D. 1994. Clonal expansion and persistence of human T cells specific for an immunodominant myelin basic protein peptide. *J. Immunol.* 152:5581.
25 Vandevyver, C., Mertens, N., van de Elsen, P., Medaer, R., Raus, J. and Zhang, J. 1995. Clonal expansion of myelin basic protein-reactive T cells in patients with multiple sclerosis: restricted T cell receptor V gene rearrangements and CDR3 sequence. *Eur. J. Immunol.* 25:958.
26 Vandenbark, A. A., Chou, Y., Whitham, R., Mass, M., Buenafe, A., Liefeld, D., Kavanagh, D., Cooper, S., Hashim, G. and Offner, H. 1996. Treatment of multiple sclerosis with T-cell receptor peptides: results of a double-blind pilot trial. *Nat. Med.* 10:1109.
27 Yewdell, J. W. and Bennink, J. 1999. Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. *Annu. Rev. Immunol.* 17:51.
28 Pamer, E. and Cresswell, P. 1998. Mechanism of MHC class I-restricted antigen processing. *Annu. Rev. Immunol.* 16:323.
29 Kumar, V. and Sercarz, E. 1993. The involvement of T cell receptor peptide-specific regulatory $CD4^+$ T cells in recovery from antigen-induced autoimmune disease. *J. Exp. Med.* 178:909.
30 Broeren, C. P., Lucassen, M., Van Stipdonk, M., van der Zee, R., Boog, C., Kusters, J. and van Eden, W. 1994. CDR1 T-cell receptor beta-chain peptide induce major histocompatibility complex class II restricted T–T cell interactions. *Proc. Natl Acad. Sci. USA.* 91:5997.
31 Zhang, J., Vandevyver, C., Stinissen, P., Medaer, R. and Raus, J. 1995. *In vivo* clonotypic regulation of human myelin basic protein-reactive T cells by T cell vaccination. *J. Immunol.* 155:5868.
32 Zhang, J., Rivera, V., Tejada-Simon, M., Yang, D., Hong, J., Li, S., Haykal, H., Killian, J. and Zang, Y. 2002. T cell vaccination in multiple sclerosis: results of a preliminary clinical trial. *J. Neurol.* 249:212.
33 Medaer, R., Stinissen, P., Truyen, L., Raus, J. and Zhang, J. 1995. Depletion of myelin basic protein-reactive T cells by T cell vaccination: a pilot clinical trial in multiple sclerosis. *Lancet* 346:807.